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Supporting Information

One-pot one-cluster synthesis of fluorescent and bio-compatible Ag_{14} nanoclusters for cancer cell imaging

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1. Chemicals

Silver nitrate ($AgNO_3$, 99.8% metals basis, AR), Sodium borohydride ($NaBH_4$, 98% metals basis, Aladdin), Methanol (≥99.5%, AR, SCR), Sodium hydroxide (NaOH, ≥96.0%, AR, SCR), L-Reduced Glutathione (GSH, ≥98.0%, SIGMA-ALDRICH), Ethanol (≥99.7%, AR, SCR), Butyl alcohol (≥99.5%, AR, SY), *n*-Propanol (≥99.0%, AR, SCR), Hexadecyl trimethyl ammonium Bromide (CTAB, ≥99.0%, AR, SCR), F12 medium (Hyclone), Fetal calf serum (Biowest), Paraformaldehyde (≥94%, AR), Lung cancer cell A549 (ATCC). The CdTe/ZnTe and CdSe quantum dots are from CHINA BEIJING BEIDA JUBANG SCIENCE & TECHNOLOGY CO., Ltd. All chemicals were used as received without any pretreatment.

Nanopure water (resistivity 18.2 MΩ·cm) was purified by a Milli-Q (Millipore Co.) ultra-pure water system. All glass containers and stir bars were thoroughly cleaned with aqua regia ($HCl:HNO_3$ = 3:1 vol), rinsed with an abundant amount of Nanopure water, ultrasonic clean for several minutes, and then dried in an oven prior to use.

2. Synthesis of Ag Clusters (or nanoparticles or complexes)

$Ag_{14}(SG)_{11}$ Clusters: The synthetic method was referred to our previous work.[1] First, the silver salt ($AgNO_3$, 0.0849 g, 0.5 mmol) was dissolved in 25 ml water; the solution was kept at ~0°C in an ice bath, and the magnetic stirring speed was ~1200 rpm. Then the thiol ligand (GSH, 0.615 g, 2 mM) was added to the cold Ag solution to form the silver-GSH precursor. The pH value of the reaction solution was adjusted to ~7 (using 0.095 g sodium hydroxide solid and ~300 μl 0.5 M NaOH aqueous solution) until the white precipitate completely disappeared. To this clear solution, 5 ml of ice-cold aqueous $NaBH_4$ (0.189 g, 5 mM) were slowly added dropwise under vigorous stirring. After ageing for over 7 hours, the clusters

were precipitated with methanol. The collected solid can be further purified by recrystallization from MeOH/H_2O solution.

8nm Ag nanoparticles: First, the silver salt ($AgNO_3$, 0.0849 g, 0.5 mM) was dissolved in 25 ml water; the solution was kept at room temperature, and the magnetic stirring speed was ~1200 rpm. Then the thiol ligand (GSH, 0.1538 g, 0.5 mM) was added to the Ag solution to form the silver-GSH precursor. To this solution, 5 ml of aqueous $NaBH_4$ (0.189 g, 5 mM) were slowly added dropwise under vigorous stirring. After ageing for over 2 hours, the clusters were precipitated with methanol. The collected solid can be further purified by recrystallization from MeOH/H_2O solution. For the UV-Vis and TEM characterization, see **Figure S15**. The Ag/S atomic ratio revealed by quantitative XPS measurement is 3.0:1.

Ag-GSH complexes: First, the silver salt ($AgNO_3$, 0.0849 g, 0.5 mM) was dissolved in 25 ml water; the solution was kept at ~0°C in an ice bath, and the magnetic stirring speed was ~1200 rpm. Then the thiol ligand (GSH, 0.615 g, 2 mM) was added to the cold Ag solution to form the silver-GSH precursor. The pH value of the reaction solution was adjusted to ~7 (using 0.095 g sodium hydroxide solid and ~300 μl 0.5 M NaOH aqueous solution) until the white precipitate completely disappeared. The complexes were precipitated with methanol. The Ag/S atomic ratio revealed by quantitative XPS measurement is 0.80:1.

3. Cell-imaging

For cell imaging, lung cancer cells (A549) were cultured in F12 medium with 10% fetal calf serum at 37 °C under 5% CO_2. 100 μg/mL clusters dispersion was added to the cells and allowed to incubate for 24h. The unbound clusters were washed away with PBS and cell samples were fixed with 2% paraformaldehyde solution.

4. Characterization Methods

(1) UV-Vis spectroscopy: UV-Vis absorption spectra of Ag nanoclusters in water were recorded in standard quartz cuvette using a UV2550 ultraviolet and visible spectrophotometer (Shimadzu, Japan).

(2) Polyacrylamide gel electrophoresis (PAGE): The PAGE experiments of Ag clusters were conducted at ambient temperature, using homemade polyacrylamide gels with various concentration, in tris(hydroxymethyl)aminomethane base buffer of a pH~8.

(3) Mass Spectrometry (ESI-MS): ESI-MS analysis was conducted on the Waters Xevo G2-S Qtof. The sample was dissolved in pure water with a final concentration of 10 mg/ml. The spray voltage was set at 2.0 kV and the cone voltage was set at 20 V. The cone gas flow rate was set at 50 L/h and the desolvation gas flow rate was set to 800 L/h. The source temperature was set at 100 °C and the desolvation temperature was set at 350 °C.

(4) Nuclear magnetic resonance (NMR): The NMR spectrum analysis was obtained on an AVANCE AV 400 (Brucker, Switzerland) spectrometer.

(5) X-ray photoelectron spectroscopy (XPS): XPS analysis was conducted on a VG ESCALAB-MK photoelectron spectrometer with a Mg Kα source. All binding energies were calibrated using the C(1 s) carbon peak (284.8 eV).

(6) Transmission electron microscopy (TEM): TEM images of $Ag_{14}(SG)_{11}$ clusters were obtained on JEOL2010 at 200 keV.

(7) Fluorescence spectroscopy: Fluorescence analysis was performed on FluoroMax-4 spectrofluorometer (HORIBA Jobin Yvon, France).

(8) Fluorescence lifetimes: Lifetime measurement was determined using a picoseconds time-resolved fluorescence apparatus. The excitation laser pulses was supplied by an optical parametric amplifier (OPA-800CF, Spectra Physics), and it was pumped by a regenerative amplifier (Spitfire, Spectra Physics). The excitation pulse energy was ~100 nJ/pulse and the repetition rate was 1 KHz which focused onto a spot of 0.5 mm (diameter). The photoluminescence collected with the 90° geometry was dispersed by a polychromator (250is, Chromex) and a photon-counting type streak camera (C5680, Hamamatsu Photonics) was used for collection. The data detected by digital camera (C4742-95, Hamamatsu) and analyzed with HPDTA software. The temporal resolution was 2-100 ps and the spectral resolution was 2 nm. The nonlinear least-square fitting was used for analysis the kinetic traces from time-resolved spectra to a general sum-of-exponentials function after deconvolution of instrument response function. All of the analyses were carried out at room temperature.

(9) Laser scanning confocal microscopy (LSCM): The cells were scanned in bright field and excited at 405/488 nm using laser scanning confocal ZEISS LSM710.

(10) Fluorescence microscope: Olympus BX60.

5. Supporting Figures S1-15

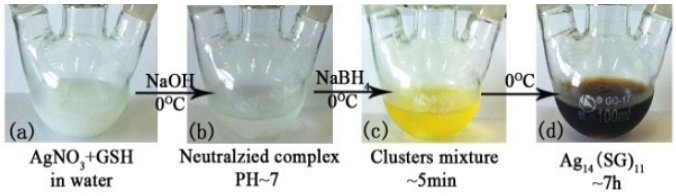

Figure S1. Color evolution during the synthesis of Ag_{14} clusters. (a) the initial $AgNO_3$-GSH complex; (b) the suspension turns to clear after the pH value was adjusted to ~7; (c) 5 min. after addition of $NaBH_4$; (d) 7h. after addition of $NaBH_4$.

Figure S2. PAGE evolution with the prolonging of reaction time from 1h to 8h (recorded per hour). It indicates the product is of high purity even without any purification after 7 hours' ageing.

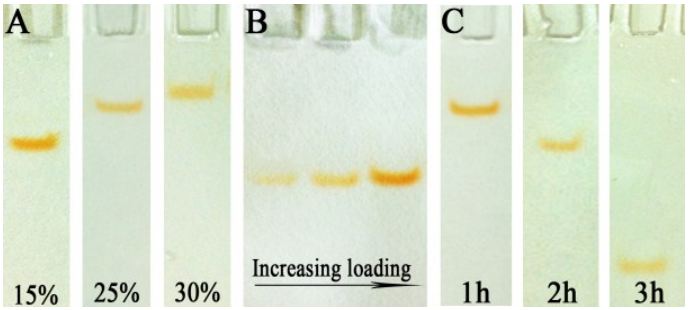

Figure S3. PAGE analysis of the purified Ag_{14} clusters in different conditions: (A) acrylamide gels with different monomer concentrations; (B) with increasing loading amount of Ag clusters from left to right; (C) different running time.

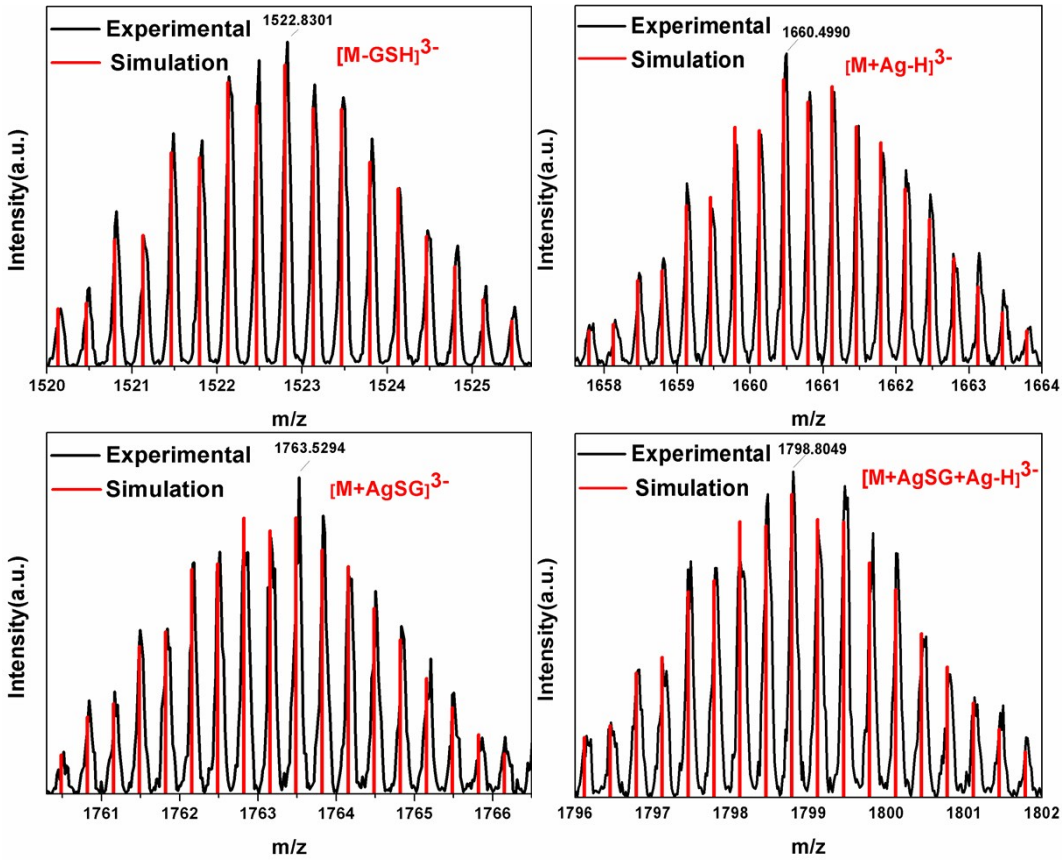

Figure S4. Isotope peaks of the $[M\text{-}GSH]^{3-}$ at 1522.8301 (deviation 0.0329), the $[M+Ag\text{-}H]^{3-}$ at 1660.4990 (deviation 0.0415), the $[M+AgSG]^{3-}$ at 1763.5294 (deviation 0.0438) and the $[M+AgSG+Ag\text{-}H]^{3-}$ at 1798.8049 (deviation 0.0202). Note: M = $[Ag_{14}(SG)_{11}\text{-}4H]$.

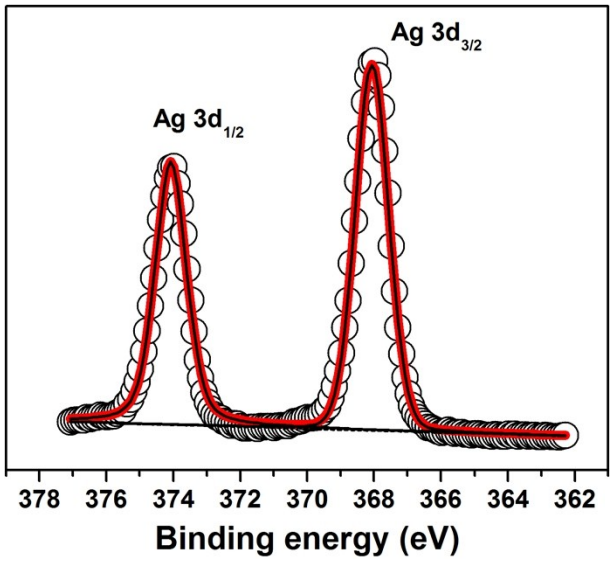

Figure S5. XPS high-resolution scan of Ag_{14} nanoclusters in the Ag 3d region.

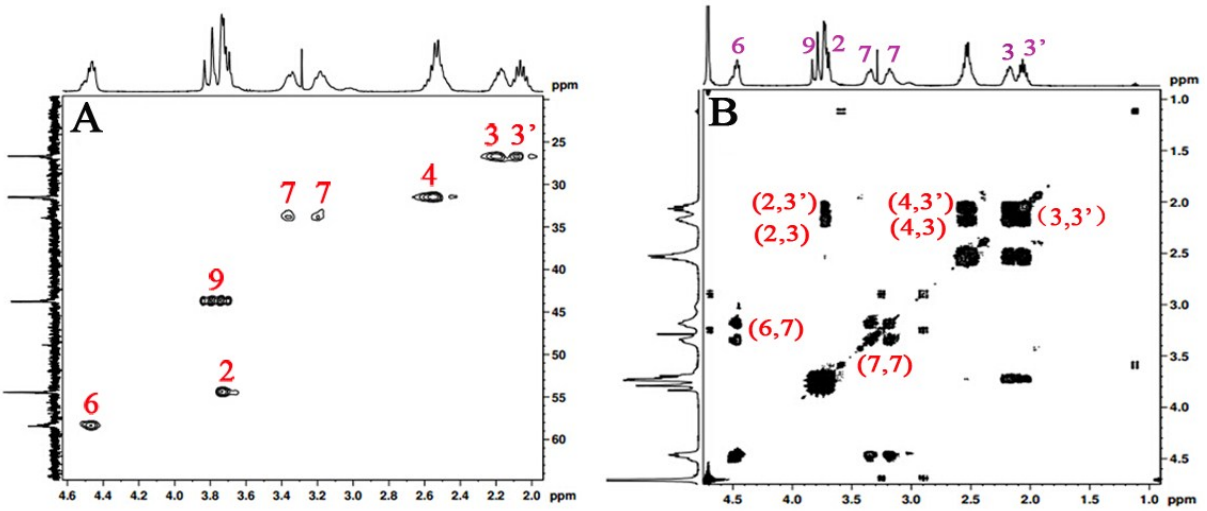

Figure S6. 2D NMR spectra of $Ag_{14}(SG)_{11}$ clusters. (A) Edited mode HSQC spectrum (B) COSY spectrum. Solvent: D_2O.

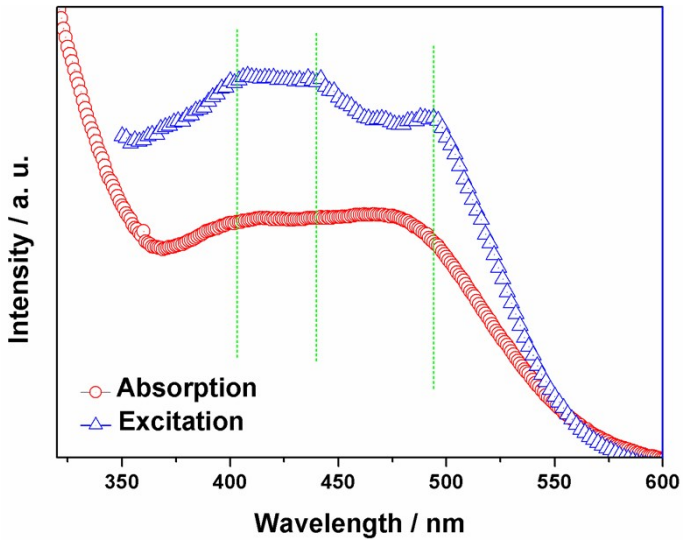

Figure S7. Absorption and excitation (monitored at 640 nm) spectra of Ag_{14}.

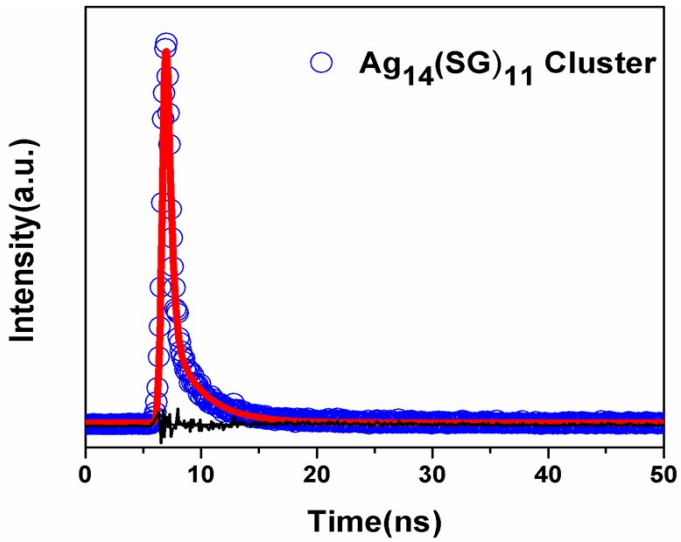

Figure S8. Lifetime measurement of the Ag_{14} clusters.

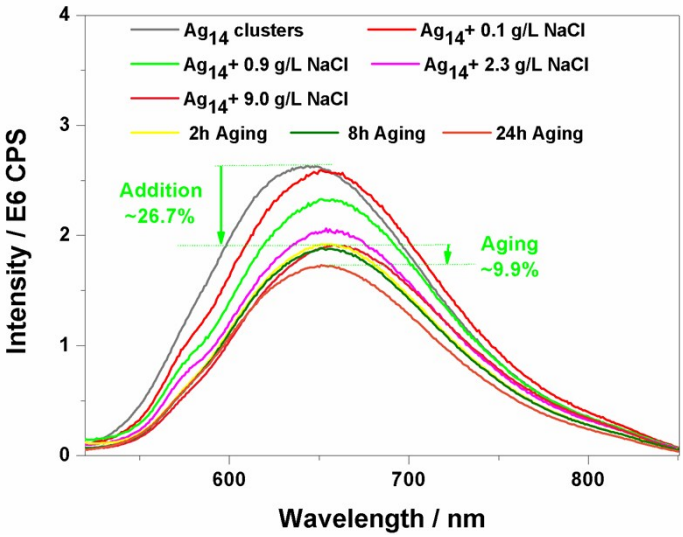

Figure S9. Fluorescence of Ag_{14} in presence of variously concentrated NaCl (0.1 g/L, 0.9 g/L, 2.3 g/L and 9.0 g/L). When the concentration was fixed at 9.0 g/L, the fluorescence was investigated after aging different time (2, 8, 24 hours); while in other cases, the fluorescence was measured immediately after the salt was added.

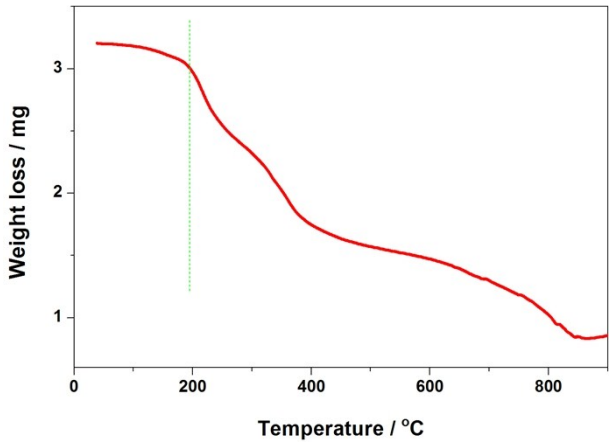

Figure S10. The TGA of Ag_{14}.

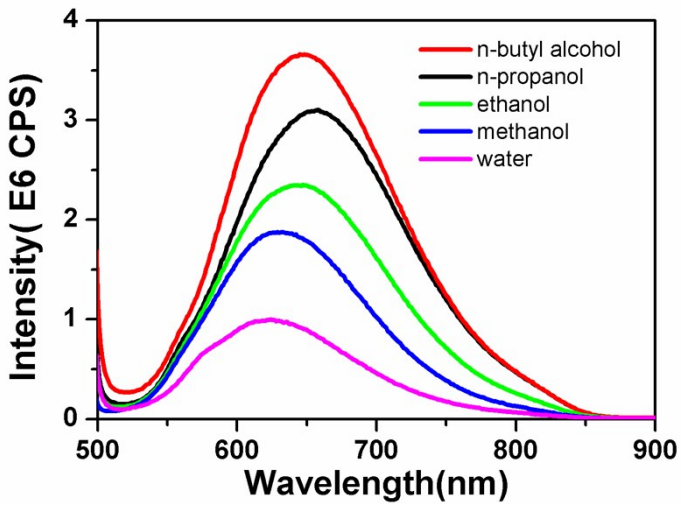

Figure S11. Solvent-dependent fluorescence of Ag_{14}.

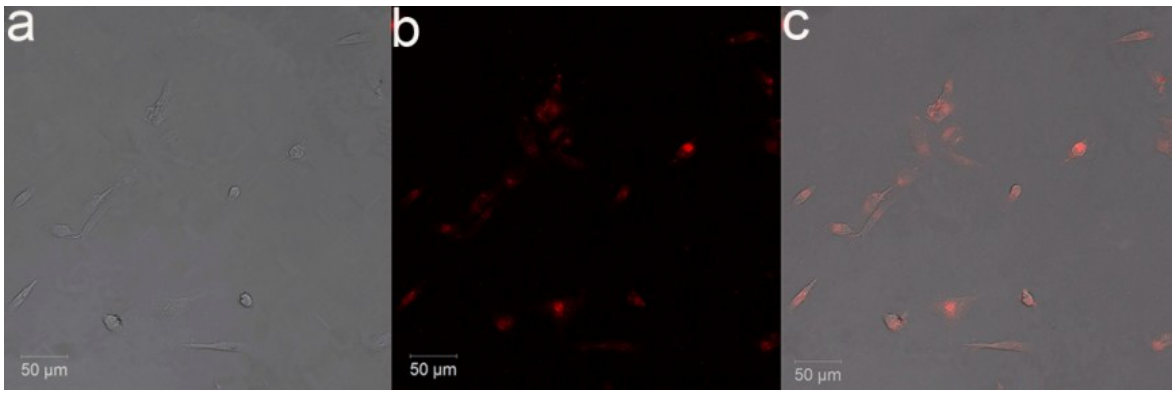

Figure S12. (a) The bright-field image of lung cancer cells incubated with $Ag_{14}(SG)_{11}$clusters for 24h; (b)TPF image (false color) of lung cancer cells incubated with $Ag_{14}(SG)_{11}$ clusters upon excitation at 405/488 nm; (c) The overlaid picture of corresponding bright-field image (a) and the TPF (b).

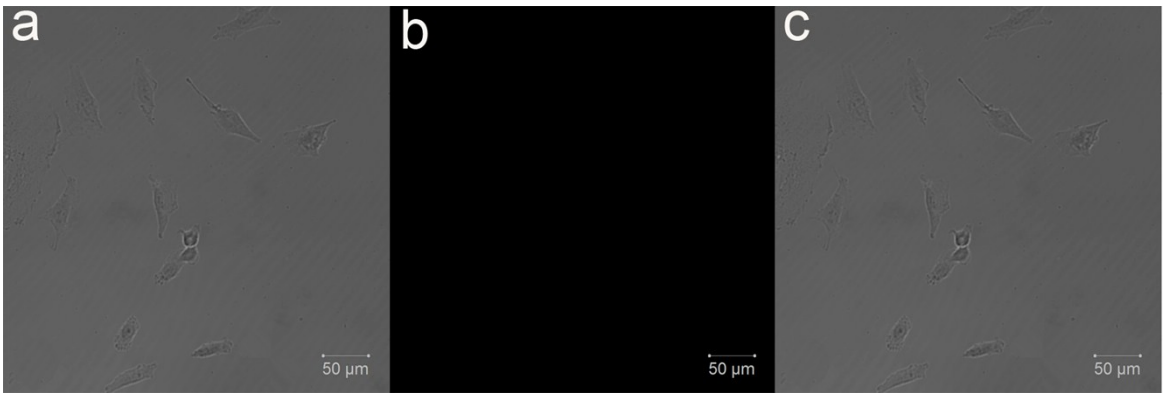

Figure S13. (a) Bright-field image of A549 cells; (b) TPF image of lung cancer cells upon excitation at 405/488nm; (c) Overlay of (a) and (b).

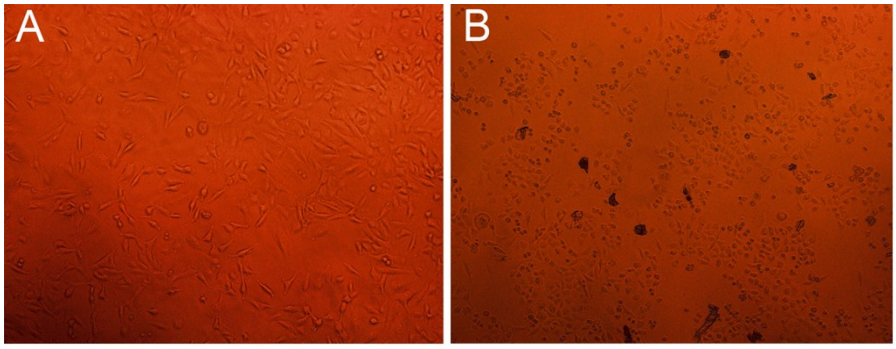

Figure S14. Fluorescence microscope images (false color) of A549 cells and A(L) cells incubated with Ag_{14} for 24h.

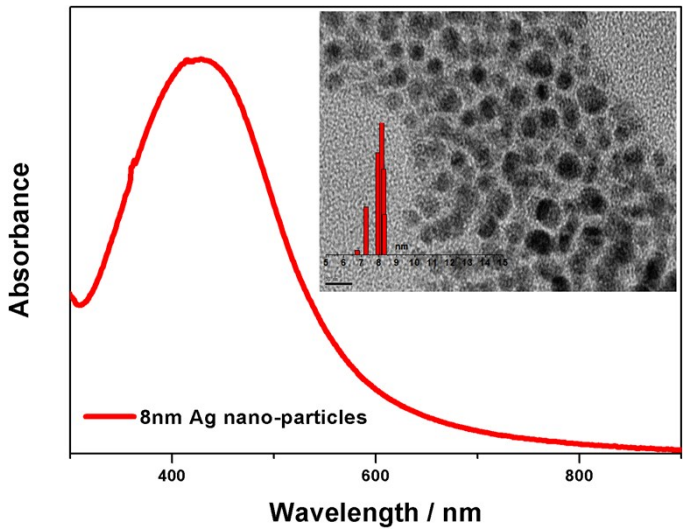

Figure S15. UV-Vis absorption spectrum of 8nm silver nanoparticles. Inset: TEM image of Ag clusters. The scale bar is 10 nm and the statistical size of nanoparticles is 8.03, ~8nm

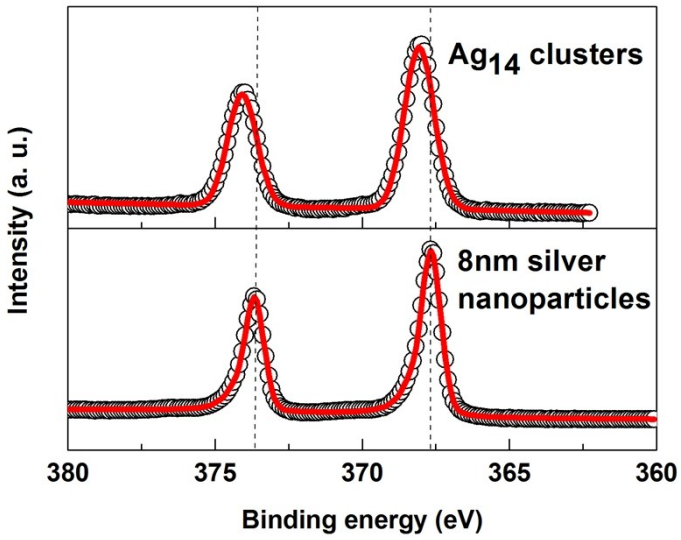

Figure S16. Comparation of Ag3d binding energies of the Ag_{14} and 8nm nanoparticles.

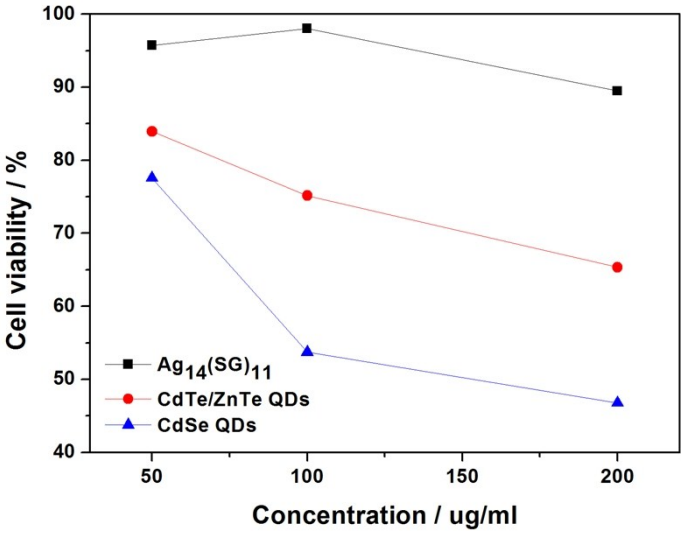

Figure S17. Viability of A549 cells in variously concentrated (50, 100, 200 ug/ml) Ag_{14} (black), CdTe/ZnTe QDs (red) and CdSe QDs (blue).

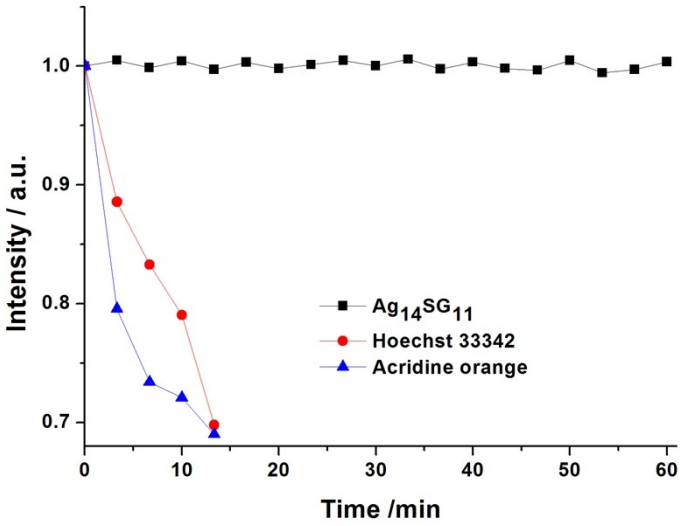

Figure S18. Photobleaching curves of Ag_{14} nanoclusters (black), Hochst 33342 (red) and Acridine orang (blue).

Reference

(1) Wu, Z.; Lanni, E.; Chen, W.; Bier, M. E.; Ly, D.; Jin, R. *J. Am. Chem. Soc.* **2009**, *131*, 16672-16674.